Should I stay or should I go?

Sandström, Cecilia


1

GENERAL INTRODUCTION AND SYNTHESIS

Preface

Barnacle geese (*Branta leucopsis*), as well as for all organisms, need to survive so they can reproduce. A key component of survival is protection against parasites and diseases, thus geese need to adapt to inferred disease risk, food availability, perceived stress, and internal factors such as wing moult and growth. The main focus of this thesis is to investigate how these factors contribute to the variation of immune activity, and to some degree health, in barnacle geese. Immunological assays were conducted on blood of geese collected from the Svalbard archipelago, Arctic tundra in Russia, wetlands in Denmark, and evergreen grass fields in The Netherlands in order to address the following questions:

i) What is the role of migratory geese in transmitting diseases between their temperate wintering areas and Arctic breeding grounds? (Chapter 2)

ii) How do ultimate (reproduction and wing moult) and proximate (disease risk and food availability) factors explain variation in immune activity? (Chapter 3)

iii) How does health status explain differences in growth? (Chapter 4)

iv) How do populations differ in their perception and immune response to an acute stressor? (Chapter 5)

Introduction to the immune system

Every living organism needs to protect itself from harmful agents in its environment. In vertebrates, this defence system is the immune system: a multi-layered protective network of different defence strategies involving a variety of mechanisms, each with different benefits and costs (Hanssen et al. 2004, Klasing 2004). The first layers of defence in the immune system are physical, chemical and behavioural barriers against an invading disease or parasitic organism. For example, the rapid change in pH between the stomach and the intestine provides a biochemical barrier, while preening, grooming and parasite avoidance are behavioural barriers. Skin and mucus in the respiratory tract are examples of a physical barrier. If invaders manage to pass the first layers of defence, other mechanisms are triggered, such as the innate and acquired immune responses (Murphy 2011).

The innate immune system responds immediately to an invading organism in a non-specific generic manner. Innate immunity cannot recognize earlier encounters and is considered the basal or oldest defence strategy in almost all life forms (Murphy 2011). Important functions of the innate immune system include the onset of inflammation, fever, agglutination and lysis of invaders, and other non-generic defences against foreign or abnormal cells. Furthermore, baseline levels of innate immune activity, such as concentrations of white blood cells (leukocytes) can indicate health status (Campbell 1995).

The acquired immune system, on the other hand, is specific and more complex. It is found solely in jawed vertebrates and reacts first after the invader has been recognised; consequently it is a delayed response, using an antigen-specific response based on immunological memory. The main actor within the acquired immune system is the leukocyte "lymphocyte" (Murphy 2011). A schematic overview of the innate and acquired system is presented in **Table 1.1**.

Table 1.1 Key characteristic of the two branches of the immune system: innate and acquired immunity.

Innate immune system	Acquired immune system
Response is non-specific	Pathogen and antigen specific response
Immediate max. response after exposure (minutes to hours)	Lag between max. response and exposure (days to weeks)
Cell-mediated and humoral components	Cell-mediated and humoral components
No immunological memory	Exposure leads to immunological memory
Found in nearly all forms of life	Found only in jawed vertebrates
Constitutive	Induced

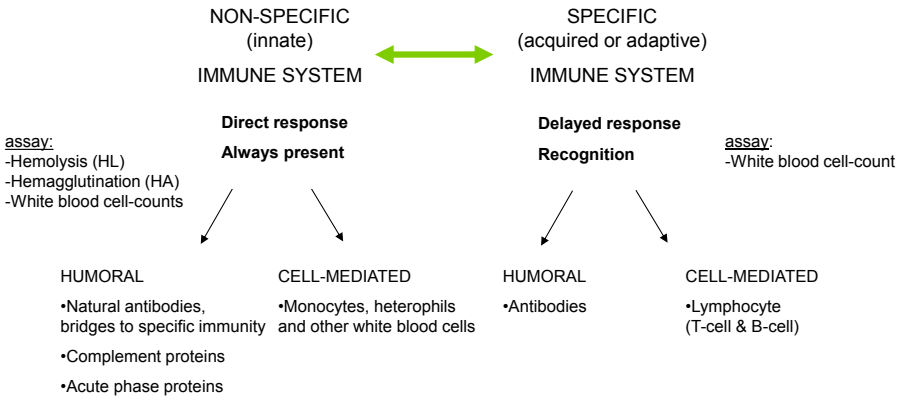

Figure 1.1 Schematic illustration of the major branches, concepts and components of the immune system relevant for this thesis. Assays used in this thesis are mentioned at the outer edges.

Both the innate and the acquired systems are subdivided into cell-mediated and humoral immunity (**Fig. 1.1**). Cell-mediated immunity deals with invaders that have entered both the body and cells of the host's body. It is very effective in removing virus-infected cells as well as fungi, cancer cells, and some protozoan organisms (Yap, Sher 1999). Cell-mediated immunity does not involve antibodies, but white blood cells, such as activated monocytes, heterophils and T-lymphocytes. Humoral immunity on the other hand, is located in the fluids of the body (excluding the blood cells) and is based on secreted antibodies, which are produced in B-lymphocytes. Also included in humoral immunity are complement and acute phase proteins. There is often redundancy between the branches and systems of immunity where different functions interact to recognize and eliminate any invader.

Box 1.1 Leukocytes and other important concepts of the immune system

Both the innate and acquired immune systems are centered on the functions of two major types of leukocytes (white blood cells): *phagocytes* and *lymphocytes*. *Phagocytes* ingest and destroy foreign invaders by phagocytosis. Since their recognition system is primitive and non-specific, they recognize and eliminate a large variety of foreign invaders and consequently, are one of the key cells in the innate immune system. Within the grouping of phagocytic cells there are two main cell types, the *monocytes* and the *polymorphonuclear granulocytes*. When a monocyte is activated it migrates from the bloodstream to other tissues, becoming a *macrophage*. Macrophages have two main functions, the *professional phagocytes,* which first engulf and then remove particular antigens, and the *antigen-presenting cells,* which capture, process and present the antigen to *T-lymphocytes*. The antigen-presenting cells are a major link between the innate and the acquired immune systems.

Another group of phagocytes are polymorphonuclear granulocytes, consisting of *heterophils* (equivalent to neutrophils in mammals), *eosinophils* and *basophils*. During early stages of inflammation, *heterophils* rapidly migrate into tissues, where they phagocytise foreign matter and die. Together with lymphocytes, they are the most abundant leukocyte circulating in the blood stream. *Eosinophils* are much less abundant, but also have the ability to phagocytise, mainly killing other cells (cytotoxic) and larger extracellular parasites. *Basophils* are the least abundant polymorphonuclear granulocytes and are only present at very low concentrations. They mediate inflammation and play a role in allergic reactions and against parasites. The precise function of *eosinophils* and *basophils* is not yet fully understood.

Lymphocytes consist of *B-lymphocytes* (B-cells) and *T-lymphocytes* (T-cells), which are responsible for the specific recognition and memory of antigens during the acquired response. Every *B-cell* is genetically programmed with a specific receptor for a particular antigen on its cell surface. When a B-cell contacts its specific antigen, it is activated and starts to differentiate into a *plasma cell* (large lymphocyte). The plasma cell produces a huge amount of the same antigen receptor molecule as the one first activating the B-cell, but in a soluble form called *antibodies*. The antibody now binds to the specific type of antigen that first activated the B-cell. When the antibody binds to its antigen, the antigen gets marked for destruction by phagocytes. When the infection is subdued, some B-cells will retain the specific antigen receptor, but will never become a plasma cell. These cells are called *memory B-cells* and are important for immunological memory and will be activated if the same antigen re-enters the body, thereby affording immunity. *T-cells* also recognize a specific antigen, but only if present on a major histocompatibility complex (MHC) molecule.

Natural antibodies are present before any immune response occurs, therefore they do not respond to a specific antigen and serve as the first response of the immune system to fight infections. As natural antibodies are not made for a specific antigen, some might bind to the antigen, whereas others do not bind at all. Once a natural antibody has bound to an antigen, however, the antibody can be modified and the "new" antibody can then bind specifically.

Haemagglutination (HA) refers to the agglutination of foreign red blood cells, the interactions between *natural antibodies* and antigens. The reaction results in clumping of the foreign material and is used as a measurement of the level of natural antibodies in the plasma.

Haemolysis (HL) reflects the interaction between *complement* and natural antibodies. Lysis refers to the process of breaking open foreign red blood cells and is a function of the amount of lytic complement proteins present in the collected blood plasma.

The *complement system* is a biochemical cascade. The system consists of a number of small proteins (complement proteins) and protein fragments, which together help to clear pathogens from the host by disrupting the pathogen's plasma membrane. The system is primarily part of the innate immune system, though it also can be recruited and activated by the acquired immune system. The system is not adaptable and does therefore not change over an individual's life time.

The *acute phase response* is associated with fever as well as loss of body mass, reduction of food intake and reduced activity (Owen-Ashley, Wingfield 2006). The response also releases *acute phase proteins* from the liver, which have antibacterial properties (Baumann, Gauldie 1994). The acute phase response is in the first line of *innate* immunity to an invader and is considered one of the most costly responses in terms of energy use, damage on the host and lost opportunities (Klasing 2004).

Different aspects of the immune system have different costs

The immune system is a highly complex and multi-layered system of overlapping and interlinked defence mechanisms. It is essential to survival and offers significant benefits, such as minimizing fitness loss caused by infections and diseases (Brown, Brown & Rannala 1995, Fitze, Tschirren & Richner 2004). However, this multifaceted and sophisticated system also comes with costs due to the energy and time it requires to develop, be activated and maintained (Schmid-Hempel, Ebert 2003, Schmid-Hempel 2003, Klasing 2004, Råberg et al. 1998). Furthermore, collateral damage to the body's healthy cells may result from certain immune responses (Råberg et al. 1998). Consequently, maximum immune activity is not always the best approach (Viney, Riley & Buchanan 2005).

Different aspects and phases of the immune system, such as the development, maintenance and utilization, have different costs (Table 1.2) (Klasing 2004). For innate immunity, the development costs of resources and time are generally low as there is no diversification or selection. Maintenance costs are moderate, since the need of replacement is low as the cells and proteins are "just circulating" while waiting for an invader. This is generally true except for the heterophils, which are short-lived white blood cells associated with inflammation (Splettstoesser, Schuff-Werner 2002, Murphy 2011). The cost of maintaining innate baseline immune activity is mainly due to resource allocation. When the innate immune system is activated, however, the costs become high. The damage to the host that are associated with inflammation, acute phase response and fever are significant, in addition to the cost of resources and lost opportunities as a consequence of sickness-related behaviour (Klasing 2004).

Induced specific immunity on the other hand is also associated with costs, but in the opposite direction. Thus, the costs of development are high (Reynaud, Weill 1996), while the maintenance, use and self-damage are low (Table 1.2) (Klasing 2004).

Table 1.2 Relationships between types and cost of the immune system during different phases (modified from Klasing 2004).

Phase	Cost	Baseline, innate	Induced, specific
Development	Time, resource	LOW-no diversification or selection	HIGH-diversification and selection
Maintenance	Resource	MODERATE- slow replacement, "resting" cells	LOW- long-lived cells, ready to be used
Use	Lost opportunities, resource	HIGH- high damage on host due to acute phase response and fever	LOW- specific response, little cell damage

Depending on the situation, such as available resources and current level of disease threat, animals can react to a threat with different immune strategies and intensities (Sheldon, Verhulst 1996). Consequently, the "best response" depends on the specific circumstances and infection status of the host (Viney, Riley & Buchanan 2005) and leads to a great variety in immune activity observed both within and among species. Exploring and explaining this variation in immune activity are central goals within ecological immunology, where measures of immunology are used to answer ecological questions, rather than understanding the precise molecular mechanisms (Sheldon, Verhulst 1996, Sadd, Schmid-Hempel 2009, Schulenburg et al. 2009).

Box 1.2 Costs and benefits of the immune system from an evolutionary and an ecological perspective

There is one major benefit of the immune system: it prevents the host from infections and diseases, and ultimately, reduces disease-related mortality. The complex system of defence mechanisms, however, does incur two types of costs to fitness: *evolutionary cost* and *ecological costs*.

Evolutionary costs are the most basic costs and are driven by natural selection (Zuk, Stoehr 2002, Schmid-Hempel 2003). Selection for different degrees or types of immune investment comes with certain consequences. For example, house sparrows (*Passer domesticus*) have a lower reproductive success if initiating an immune response (Bonneaud et al. 2003), poultry with high growth display low immune capacity (van der Most et al. 2011), and turkeys with a high egg production and a large body mass have low resistance to disease (Nestor et al. 1996). Overall, birds with a high reproductive effort are often found with more parasites and a lower immune activity (reviewed in Knowles, Nakagawa & Sheldon 2009). Consequently, natural selection favors individuals with the best balance of costs and benefits, and optimal strategy with largest fitness. While natural selection works on evolutionary time scale, my main research focus focused on an ecological time scale.

Ecological costs associated with immune activity can be seen from three standpoints: cost of resources, cost of damage, and cost of lost opportunities. The *cost of resources* is the energy or nutrients necessary to develop, maintain, and use the immune system; resources that could have been invested in other necessities, such as breeding or migration (reviewed in Zuk, Stoehr 2002, Schmid-Hempel 2003). The *cost of damage*, or immunopathology occurs when immune activity harms the host simultaneously when defending against a pathogen (Råberg et al. 1998). Thus, a balance must be maintained where the immune system is not too active, but still active enough (Reeson et al. 1998, Råberg et al. 1998, Klasing 2004). The *cost of lost opportunities* is incurred when important life-history activities such as migration, breeding or moult, are temporarily delayed while the host attempts to fight infections and/or parasites (Sanz et al. 2004, Owen-Ashley, Wingfield 2006, van Gils et al. 2007, Männiste, Hõrak 2011, Romano et al. 2011).

Understanding the concept of 'ecological immunology'

Ecological immunology is a relatively young field (Martin et al. 2011), with its roots in sexual selection and life-history theory. The functionally diverse immune system according to life-history theory is costly and thus subject to trade-offs between other life-history processes such as growth and reproduction (Sheldon, Verhulst 1996, Norris, Evans 2000). As the benefits of the immune system promote survival, hence indirectly promoting future reproduction, the immune system is likely to have evolved concomitant to other life-history traits also maximizing fitness. This initial approach of ecological immunology, based on the trade-off of costs, has resulted in various studies exploring the variation in immune activity in relation to reproductive success, growth or other physiological traits associated with fitness (Bonneaud et al. 2003, Schmid-Hempel 2003, Deerenberg et al. 1997, Verhulst, Riedstra & Wiersma 2005, Moreno-Rueda 2010b, van der Most et al. 2011).

A second and more recently developed approach emphasizes the benefits of the immune system in light of the variation found in immune activity. As the most obvious benefit of the immune system is resistance against infections, diseases, and parasites, it is easy to embrace the idea that an animal living in an environment with high disease risk possibly benefits more from an active immune system than an animal living in an environment where disease risk is low (Lindström et al. 2004, Tschirren, Richner 2006, Horrocks, Tieleman & Matson 2011). Habitats typically considered to have high disease risk are often wetter, such as mesic or tropical areas (Guernier, Hochberg & Guegan 2004, Møller 1998). Areas with a harsh or dry climate, such as the cold and windy Arctic (Greiner et al. 1975, Bennett, Montgomerie & Seutin 1992, Piersma 1997) or hyper-arid deserts (Horrocks et al. 2012a), are often categorised as low disease risk-habitats.

Barnacle geese - Migration, or not?

Many animals alternate between high and low disease risk areas during the course of annual migrations, which is reflected in the variation of their immune indices (Buehler, Tieleman & Piersma 2010b). Migratory behaviour of animals may lower disease risk (Altizer, Bartel & Han 2011) through the loss of infected individuals at the beginning of migration, in that infected individuals might simply not be able

to migrate (e.g. van Gils et al. 2007). Furthermore, migration can allow the host to periodically "escape" a parasite-laden environment when moving to a new habitat. This is especially true for parasites with transmission stages that can build up in the environment, such as various types of helminths. While hosts are gone, parasite numbers greatly decrease so that the migrating animals find a largely disease-free habitat when they return (Loehle 1995).

Barnacle geese (*Branta leucopsis*) typically migrate to high Arctic breeding grounds and return in autumn to temperate wintering grounds such as Scotland and The Netherlands. However, recent changes in agriculture and land use have improved the summer conditions for geese in The Netherlands (van Eerden et al. 2005, van der Jeugd et al. 2009), resulting in the establishment of a sedentary population of barnacle geese in the river deltas along the southwest parts of The Netherlands. These geese are part of a population that originated from the Arctic migratory geese that subsequently ceased migration (Black, Prop & Larsson 2014). The relatively young temperate population is genetically differentiated from the Arctic migratory populations, although the difference in genetic structure is small due to a high rate of genetic exchange (Jonker et al. 2013). Benefits of abandoning traditional migration are decreased predation risk during the migration itself (Jonker et al. 2010), shorter parental care (Jonker et al. 2012) and a stable food supply (van Eerden et al. 2005) at temperate summering grounds. Moreover, the sedentary population of geese has no dangerous and energy-demanding journey of thousands of kilometres to and from the Arctic summering grounds. Breeding in the energetically expensive Arctic, however, is rewarded by nutritious (though scarce) food plants (van der Graaf et al. 2006b, Black, Prop & Larsson 2014), concomitant with lowered inferred disease pressure relative to temperate sites. Reduced disease pressure in the extreme environment of the Arctic is largely due to the climate, which is inhospitable for micro- and macro-parasites and for parasite-transmitting organisms (Greiner et al. 1975, Bennett, Montgomerie & Seutin 1992, Guernier, Hochberg & Guegan 2004, Coulson 2007, Fierer et al. 2009).

How to infest the Arctic?

The dynamics of disease-causing agents and transmitters are likely to be influenced by interactions between the infected host, the susceptible host, the infection pathway and local environmental conditions. Even though the Arctic tundra is

considered as an environment of low disease pressure, high prevalence estimates of avian influenza have been reported from Northern molting and pre-migratory staging grounds (Ito et al. 1995, Wallensten et al. 2007). To determine if the congregation of migratory birds in the Arctic actually did result in infection, Pink-footed geese (*Anser brachyrhynchus*) were sampled over the full migratory flyway: from The Netherlands, via Denmark and Norway, to Spitsbergen, and all the way back again (Hoye et al. 2011). Results showed that infection occurred first when the geese return to temperate wintering ground in The Netherlands, probably when the wintering aggregations mix with dabbling ducks, a group often considered a natural reservoir of avian influenza virus (Nishiura et al. 2009, Hoye et al. 2011).

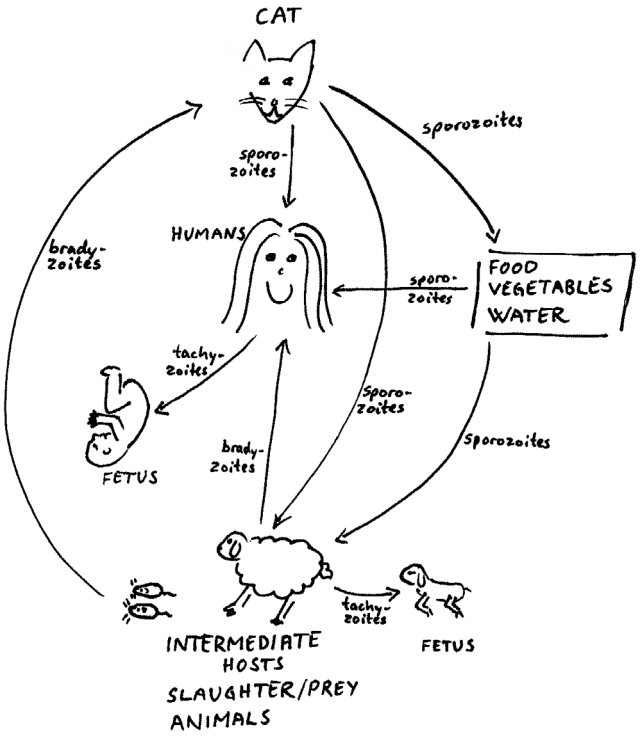

Figure 1.2 The classical life cycle and known transmission routes of T. gondii. There are three infective stages, tachyzoites (during active infection in host tissue), bradyzoites (cysts in tissue) and sporozoites (in oocysts from cat faeces). Figure is reused with permission from Prestrud (2008).

Polar regions are isolated both by their extreme environment and remote location. Nevertheless, both in the high Arctic (Prestrud et al. 2007, Oksanen et al. 2009, Jensen et al. 2010) and Antarctic (Jensen et al. 2012, Rengifo-Herrera et

al. 2012), various animal species were found to be seropositive with the parasitic protozoan *Toxoplasma gondii* even though neither area is known to harbour felines, which are the definitive host for *T. gondii* (**Fig. 1.2**). For example, in the high Arctic Svalbard archipelago (78–81°N, 10–30°E), including the main island Spitsbergen, no wild felines are present and domestic cats are prohibited. Yet, *T. gondii* infection has been observed in resident top predators such as Arctic foxes (*Vulpes lagopus*) and polar bears (*Ursus maritimus*) (Prestrud et al. 2007, Oksanen et al. 2009, Jensen et al. 2010). How is this possible?

Thesis outline

Transmission of T. gondii *by migratory birds*

Despite the absence of felids on the Arctic archipelago of Svalbard, *T. gondii* has been found in resident wildlife. Whether the initial infestation of *T. gondii* in resident wildlife is a result of oocyst transported via ocean currents or via tissue cysts from migratory animals is largely unknown. **Chapter 2** addresses:

> What is the role of migratory geese in transmitting diseases between their temperate wintering areas and Arctic breeding grounds?

It has been proposed that oocysts of the parasite could travel with the sea currents and subsequently with filter feeding fish and mussels (Arkush et al. 2003, Miller et al. 2008, Massie et al. 2010), thereby entering the high Arctic food web. Migratory birds also have been discussed as possible vectors (Prestrud et al. 2007). Together with my co-authors, I investigated this alternative pathway further: an "aerial" transmission route via migratory geese. We analysed antibody levels of two migratory geese (pink-footed, *Anser brachyrhynchus* and barnacle, *Branta leucopsis*) and three non-migratory geese (greylag, *Anser anser*, Canada, *Branta canadensis* and barnacle, *Branta leucopsis*), specifically by analysing plasma of goslings both on their hatching site and during their first year spring migration. No seropositive juvenile geese were found at any brood-rearing location, however nine-month old geese were seropositive during their first spring migration. We concluded that goose species encounter *T. gondii* infection when arriving in their wintering areas for the first time. But how does the parasite infect Arctic resident

top predators? The following summer the infected geese transport the parasite to their Arctic summering grounds. When an infected goose is consumed by an Arctic predator/scavenger, the parasite enters the Arctic food web.

We also found that infected geese showed a high degree of interannual variation in their levels of seroprevalence. This was surprising as it is generally expected that infected individuals remain protected against the parasite with specific antibodies circulating in the blood stream such that upon re-infection, circulating antibody concentrations can rapidly increase and stop the invader. To our surprise, we found that almost half of the seropositive birds showed no seroprevalence the following year. Such annual variation of seroprevalence of antibodies against *T. gondii* was previously unknown. By extension our study suggests that other aspects of the immune system may display temporal variability. Hegemann et al. (2012a) showed that the immune activity of skylarks (*Alauda arvensis*) varies between years and between annual cycle events, such as breeding and migration. They suggested that birds seasonally modulate their immune system, and that this modulation is not only a trade-off between energetically-demanding predictive annual-cycle demands (e.g. breeding, moult, wintering or migration) but that environmental variation, such as food availability and disease pressure is equally important (Hegemann et al. 2012a).

Temporal and spatial variation in immune activity and health

For some species, such as the barnacle goose, variation in these environmental factors is expected to vary not only between, but also within an annual cycle event. This is especially true during wing moult when geese are restricted in movement and hence confined to a certain area. Accordingly, **Chapter 3** was designed to better understand the influence of physiological demands (internal factors, such as wing moult) and environmental circumstances (external factors, such as disease and food) on the immune system within the annual cycle event. Specifically:

> How do ultimate (reproduction and wing moult) and proximate (disease risk and food availability) factors explain variation in immune activity?

We focused on variation in immune activity within one life-cycle event. Variation in immune defence in birds is often explained by external factors such as food availability and disease pressure or by internal factors such as moult and

reproductive effort. In **Chapter 3**, these factors were explored together in one sampling design by measuring immune activity over the time frame of the (wing) moulting period of Arctic-breeding barnacle geese.

During moult, energetic costs are associated with feather growth (Owen, Ogilvie 1979, Lindström, Visser & Daan 1993, Silverin et al. 1999, Black, Prop & Larsson 2014), increased thermoregulation due to lost feather-insulation (Hohman, Akney & Gordon 1992, Klaassen 1995), and shifts in somatic tissues towards strongly developed leg muscles during the flightless moulting period (Fox, Kahlert 2005). If baseline immune activity is mainly affected by internal factors such as moult, variation in immune activity is expected to be strongly associated with the stage of feather growth. On the other hand, variation in immune activity could be explained by external factors, such as disease pressure and food resources.

Geese are constrained in their mobility during wing moult, and as a result local food resources are depleted as the season progresses (Black, Prop & Larsson 2014). In addition, as the birds intensively graze the tundra and repeatedly use the same grazing areas, the risk of cross-infection is likely to increase. If this is the case and if immune activity is mainly associated with external factors, then immune activities among individuals would be synchronised and there should be a strong association between immune activity and calendar date, where calendar date reflects temporal changes in the external environment.

In **Chapter 3,** my co-authors and I show that the variation in immune activity during wing moult in migratory barnacle geese is strongly associated with calendar date and to a smaller degree with the growth of the wing feathers. To further explore this environmental factor, we compared the migratory population in Svalbard (Arctic) with the sedentary population in The Netherlands. As immune activity in the Arctic population was mainly determined by external factors, including disease pressure, a difference between the immune performances between these two populations was expected, as the environments differ. Indeed, we found that the immune activity in the Arctic population was generally lower than in the temperate population, where both disease pressure and food availability was predicted to be higher (**Chapter 3**).

However, as immune activity is increased in environments of inferred higher disease pressure (Buehler, Piersma & Tieleman 2008, Buehler, Tieleman & Piersma 2010b, Horrocks, Matson & Tieleman 2011, Horrocks et al. 2012a, Horrocks et al. 2015) the temperate population might have lower health status than the Arctic population. Therefore, **Chapter 4** asks:

| How does health status explain differences in growth?

To answer this question, 16 barnacle geese goslings were hand-raised in the Arctic Svalbard and another 16 in the temperate Netherlands in order to monitor the effect of health status and parasite load on growth (**Chapter 4**). A full post-mortem examination of the fledging goslings revealed that the temperate group had: 1) a lower overall health status, 2) a significant higher burden of intestinal and renal parasites, 3) clear histopathological changes in liver, kidney and intestine, and 4) significantly lower body mass. These results are consistent with other studies demonstrating a clear negative correlation between growth and parasite burden (Chappell, Zuk & Johnsen 1996, Tompkins, Greenman & Hudson 2001, Dæhlen 2003, Dudaniec, Kleindorfer 2006, Fessl, Kleindorfer & Tebbich 2006, Quiroga, Reboreda 2012).

To determine if health status explained the lower growth rate, we treated half of the Arctic group with an anthelmintic (drug used to expel parasitic worms) to experimentally improve the health status. Within the Arctic group we found no effect of the treatment on growth. In The Netherlands the equivalent treatment was not approved by authorities, nevertheless, the natural span in body mass and health status was relatively broad. However, we found no causal link between growth and health status within the temperate group. We concluded that: 1) variation within our groups was too small to identify an effect of health status on growth and 2) there are other factors of importance affecting growth in young geese.

Together, the past and the present shape future immune activity

So far it has been shown that immune activity (**Chapters 2** and **3**) and health status (**Chapter 4**) differ with habitat and that differences in the environmental factors are the main cause of these findings. The combination of previous experiences (e.g. **Chapter 2**), type of infection, the condition of the host and its current environment (e.g. **Chapter 3**) determine the impact of an infection on the host (Martin, Hawley & Ardia 2011). Disease-causing agents are known to have an effect on the immune activity (Horrocks, Matson & Tieleman 2011, Murphy 2011, Horrocks et al. 2012a, Horrocks et al. 2015), and encounters with these agents are likely to be similar within one population. Response to stress, both chronic and acute, is also known to have a significant effect on immune activity (Dhabhar 2014, Dhabhar 2009). For example, handling of wild animals causes an acute stress response, resulting in: 1) elevated stress hormones (Reneerkens et al. 2002, McEwen, Wingfield 2003, Millet

et al. 2007, Buehler, Piersma & Tieleman 2008), 2) an altered immune response (McEwen et al. 1997, Ewenson, Zann & Flannery 2003, Berzins, Tilman-Schindel & Burness 2008) and 3) altered baseline immune parameters (Apanius 1998, Scope et al. 2002, Buehler et al. 2008a). Overall, a clear, but not always uniform, relationship between experienced stress and immune activity has been documented (Matson, Tieleman & Klasing 2006, Martin 2009, Dhabhar 2009). **Chapter 5** leads to the final research question of this thesis:

> How do populations differ in their perception and immune response to an acute stressor?

In the final **Chapter (5),** my co-authors and I evaluate differences in the immune response to a common stressor by measuring the stress-related immune response after capturing wild barnacle geese. We sampled geese during wing moult in the Arctic and temperate summering grounds, over four populations differentially exposed to human presence (high, moderate and low exposure) in order to test the hypothesis that populations that are less frequently exposed to human activity are likely to exhibit a stronger stress-related immune response during capture. Results revealed that different populations of wild barnacle geese were differentially sensitive to (acute) stress, with the least human-habituated population showing the strongest changes in immune activity during capture. This study emphasises the necessity to keep different aspect in mind when studying immunological variation as both short temporal scale variation (e.g. duration of capture) as well as large spatial scale variation (e.g. disease pressure or food abundance) can play a significant role.

What can we learn from my research?

One important finding of my research is, no matter how clear the effect of the environment explains the variation in immune activity, at the end of the day it is the state of the single individual that determines the actual impact of the environment. This is most strongly demonstrated in **Chapter 4**, by hand-raising goslings in two different environments. The temperate sample was significantly infested with parasites, though we could not find a causal link between health status and growth. The smallest (body mass) individual might well have been the

smallest due to the energy invested in its immune system and defence, or simply due to its infestations. In **Chapter 2** my co-authors and I show that infection of the parasite *T. gondii* likely occurs at temperate wintering grounds, subsequently entering the Arctic ecosystems with its migratory goose-host. It also is clear that the variation in immunity is greater than expected, where the (detectable) levels of specific antibodies is not as constant as assumed before. The levels of circulating antibodies are most likely correlated to its current benefits, ready to rise upon re-infection at temperate wintering grounds. This leads to my second conclusion: the benefits of immune activity are important when explaining its variation. As geese are restricted in movement during wing moult, inferred increase of disease pressure at moulting grounds likely increase the benefits of an active immunity, as it most likely contributes to a higher fitness (**Chapter 3**). We suggest in **Chapter 3** that proximate factors, such as disease risk and food availability, are important when explaining variation in immune activity. Consequently, I agree with Horrocks (2012) and Hegemann (2012) in calling for a wider focus when explaining variation in immune activity, not exclusively on costs, but also to include the benefits of an active immune system. Disease risk differs between habitats; for example pink-footed geese get infected with avian flu first when being on temperate wintering grounds (Hoye et al. 2011). Disease pressure is important in shaping the immune activity; however it is not the only factor. As mentioned before, the state of an individual is of relevance, moreover how the individual perceive its environment. The same stressor can cause different immune-stress responses between different individuals and populations (**Chapter 5**). When populations differ in their perception and immune response to acute stress, immune measures become difficult to interpret and a holistic view of the studied system is essential to bear in mind.

Abandoning migratory traditions - a flexible non-migrant

Last but not least, I must acknowledge the barnacle goose for its flexibility. How can one explain that the barnacle goose has adapted to a changing environment, while none of the other Arctic breeding and temperate wintering species of geese have? For example, greater white-fronted geese (*Anser albifrons*) winter in The Netherlands and just as for the barnacle goose, some pairs abandoned northwards migration in the 1980's and began to breed in the Dutch river deltas. However,

the population of greater white-fronted goose never showed a similar dramatic increase as a result of this new strategy and has remained small (Koffijberg, van Winden 2013). Pink-footed geese (*Anser brachyrhynchus*) also winter in The Netherlands, but has kept its migratory traditions and breeds solely in the high Arctic (Madsen et al. 1999, Koffijberg, Foppen & van Turnhout 2013). Why it's only the barnacle goose that has been successful in expanding its breeding habitat to temperate grounds is an unanswered question. What is obvious is that they are successful (Boele et al. 2016, van der Jeugd, Kwak 2017). Despite new management plans, such as using disturbance programs where they are not welcome, trying to concentrate the population to areas where the (economic) damage is minimal, regulating the population by culling and removing eggs from nests, the Dutch barnacle goose population continues to increase.

Future management of the increasing barnacle goose population in The Netherlands should be re-examined. For example, forced dispersal programs inevitably result in geese returning to the same area. With few sufficiently large feeding grounds, the geese are left with little choice other than to return to the land from which they were dispersed. In addition, the observed variation in reaction to acute stress (**Chapter 5**) suggests that the geese adapt to the stressor as they may accommodate to the disturbance. It is clear that the Dutch breeding population of barnacle geese has continued to increasing (Boele et al. 2016, van der Jeugd, Kwak 2017) despite the disturbance program and a low health status (**Chapter 4**). Previous studies on barnacle geese have suggested that the high predation pressure over the migratory fly-way has contributed to the emergence of the temperate non-migratory population (Jonker et al., 2010). Will "predation by parasite" be the next major hurdle or will the population continue to grow as long as the geese are rewarded with a stable food supply and a minimum of large predators?

One of the underlying questions of this thesis was: Can health issues determine the population size and distribution of the barnacle goose? That is a question I haven't been able to answer. Health issues may determine the population size of barnacle geese especially if disease rapidly permeates the populations. However, this hypothesis may be tempered by the capacity of the barnacle geese to adjust its immune activity to the current level of disease pressure, coupled with the plasticity to adjust to local stressors. Regardless, it is remarkable that the barnacle goose is the only one of the traditionally Arctic migratory geese species that has altered its traditional migration behaviour by establishing a breeding population in The Netherlands, an area that previous was used solely as wintering ground.